*BIOSYNTHESIS OF β-D-GALACTOSIDASE CONTROLLED BY PHAGE-CARRIED GENES. III: DEREPRESSION OF β-D-GALACTOSIDASE SYNTHESIS FOLLOWING INDUCTION OF PHAGE DEVELOPMENT IN LYSOGENIC BACTERIA**

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When *E. coli* bacteria carrying the prophage λ*dg*, which contains the *gal* loci for galactose utilization, are treated with doses of ultraviolet (UV) light sufficient to induce phage production in λ-lysogenic bacteria, the *gal* genes become derepressed.[1] That is, synthesis of galactokinase in the absence of an external inducer increases 20- to 30-fold over the constitutive level. A similar result has been reported for galactose-1-phosphate uridyl transferase.[2] Following UV induction, even *gal*$^+$ bacteria that carry a normal λ prophage show a marked constitutive synthesis of these enzymes,[1] as though the induction of prophage λ, which is attached in the immediate vicinity of the chromosomal *gal* genes,[3] caused derepression of these genes.

A similar derepression by z^+ genes in prophage P1*dl* was independently observed two years ago in our laboratory. In the course of the production of high-frequency-transducing (= HFT) lysates by UV treatment of heterogenote *E. coli* lac^{del} (P1*dl* $i^+ z^+$) it was noted that the crude lysates, which were prepared in media without external inducers, contained significant amounts of β-D-galactosidase, while the unirradiated bacteria had much less. A systematic study of this phenomenon was undertaken following the discovery of the escape phenomenon described in the preceding paper,[4] and the results are reported here.

Materials and methods were as described in a previous paper.[5] UV treatment was done on bacteria collected from growing cultures and resuspended in saline. After irradiation the bacteria were diluted in TGA medium.

Results.—Derepression following UV irradiation: Figure 1 illustrates the production of β-D-galactosidase and its release in extracellular form by a culture of *E. coli* $i^+ z^- y^+$ (P1*dl* $i^- z^+$) following UV treatment and P1-superinfection. Also shown is the production of transducing activity assayed in samples from the same culture. There is a close parallelism between release of transducing phage and of galactosidase; both are presumably freed by bacterial lysis. A delay occurs between synthesis and release of enzyme, so that the total amounts of enzyme are at first in excess over the cell-free enzyme. Ultimately, however, practically all the enzyme formed is released as free enzyme. This is true even with UV treatment alone without superinfection with P1, and indicates that all bacteria in which enzyme is produced as a response to UV are ultimately lysed.

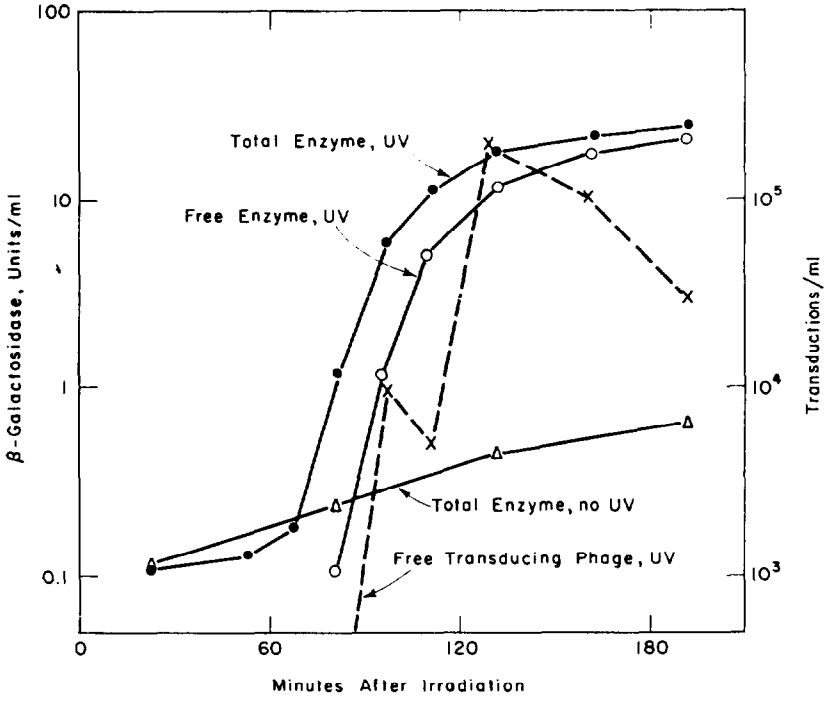

FIG. 1.—Production of galactosidase and of transducing phage after UV irradiation of the heterogenote *E. coli* strain 3.0S0-13-4-1, i^+z^-(P1*dl* i^-z^+). The amount of enzyme in a nonirradiated culture is also shown. No inducer was present.

Figure 2 shows the effect of varying UV doses on the amounts of enzyme and of phage produced. For any given strain the optimal dose is the same for both effects, indicating that they result from a common mechanism. With the strain used in the experiments of Figure 2, the maximum amount of galactosidase formed corresponds to what would be produced if about 5 to 10 per cent of the bacteria had been either genetically constitutive or grown with an inducer.

Table 1 provides a comparison of the amounts of enzyme synthesized constitutively by a variety of strains after UV treatment. The significant findings are as follows: (*a*) UV treatment provokes constitutive β-galactosidase synthesis only when the z^+ gene is in the P1*dl* prophage, not when it is in the bacterial chromosome, even though a normal P1 prophage may be present (at variance with the findings with the λ system[1,2]); (*b*) the amount of UV-initiated enzyme synthesis is different in different strains, being generally higher for strains that carry a P1*dl* i^-z^+ prophage; it is in all cases much less than the full TMG-induced amount would be.

The UV treatment that causes derepression in the heterogenotes carrying P1*dl* z^+ also leads to production of transducing particles of P1*dl* phage. The production of transducing phage is greatly increased, for most strains, by superinfection with active P1 after irradiation.[6] It is interesting to compare the production of galactosidase and of *lac*-transducing activity after these treatments. Representative results are given in Table 2. Clearly, UV treatment alone is responsible for the

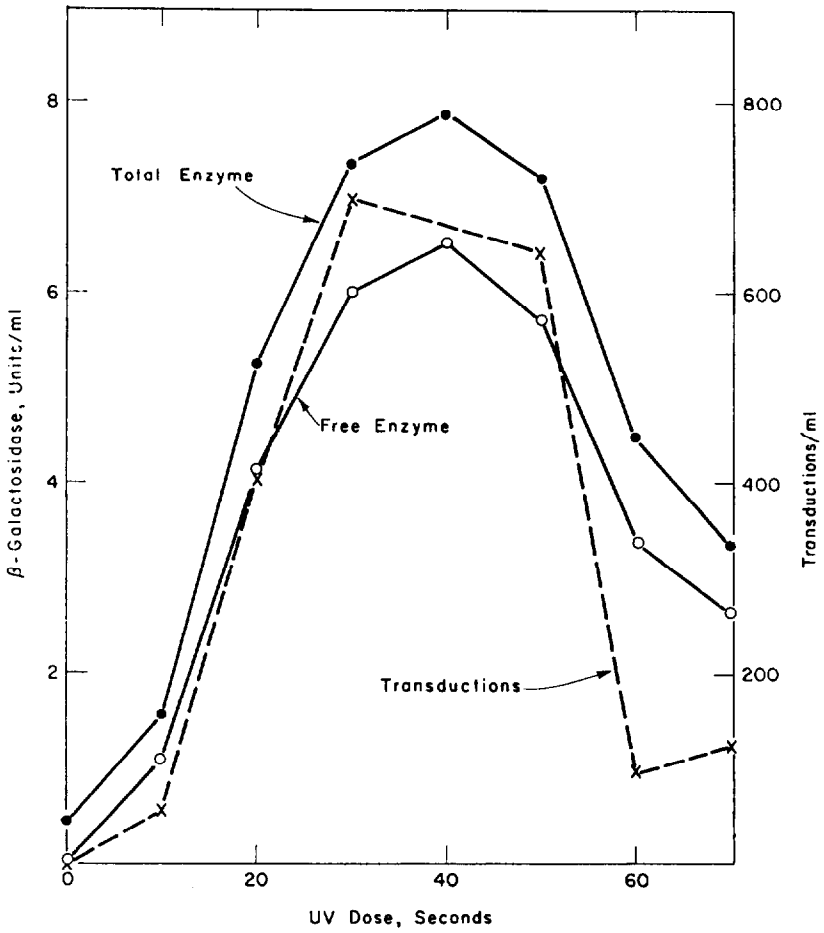

FIG. 2.—Production of galactosidase and of transducing phage by irradiated *E. coli* strain 3.0S0-13-4-1 (see Fig. 1) as a function of the UV dose. The values given are measured 200 min after irradiation. Note in Figure 1 that by this time the transducing phage titer may have decreased substantially.

derepression of enzyme synthesis; superinfection with P1, which greatly increases the production of transducing activity, does not affect enzyme production significantly.

It was also found that the amount of enzyme formed after UV-treatment depends on the medium used, being lower in TGA medium than in a tryptone-yeast extract broth, at least for some of the heterogenote strains.

The basal levels of galactosidase present in unirradiated, uninduced cultures of the heterogenotes are often higher than those of normal inducible *E. coli* strains, especially when the endogenote is i^+ and the exogenote i^-.[4] In order to check whether this base-line constitutive synthesis of enzyme was due to a small minority of i^- mutant or recombinant homogenotes, a detailed study was made of strain *E. coli* 3.0S0-13-4-2, which is $i^+ z^-$(P1*dl* $i^- z^+$) and produces constitutively 2 per cent as much enzyme as after induction by TMG. A culture of this strain was plated out on tryptone agar; isolated colonies were picked at random, grown in

TABLE 1

PRODUCTION OF GALACTOSIDASE WITHOUT INDUCER AFTER UV IRRADIATION

Bacterial strain	Chromosomal genes	P1*dl* prophage	Treatment UV	Treatment P1	Maximum E/O.D. after treatment (*a*)	E/O.D. untreated control (*b*)	Ratio (*a*)/(*b*)
3.000	i^+z^+	...	+	−	0.99	1.82	0.55
W4032 *lac*$^+$	i^+z^+	...	+	−	1.03	1.03	1.0
			−	+	1.78	0.97	1.8
			+	+	0.98	1.21	0.8
W4032 *lac*$^+$(P1)	i^+z^+	...	+	−	0.61	1.24	0.5
3.0S0-13-4-1	i^+z^-	i^-z^+	+	−	100	1.1	88
			+	+	115	1.1	101
3.0S0-13-4-2	i^+z^-	i^-z^+	+	−	99	19	5.3
			+	+	133	20	6.7
2.0S0-13-4	i^+z^-	i^-z^+	+	−	11	2.2	5.0
Sh 125-13-4	i^+z^-	i^-z^+	+	−	17	2.2	7.7
W4032-W-5	*lac*^{del}	i^+z^+	+	−	4.9	1.2	4.1
			+	+	4.5	1.1	4.3
W4032-W-1	*lac*^{del}	i^+z^+	+	−	5.5	1.4	3.9
			+	+	5.7	1.6	3.5
2.0S0-W-1	i^+z^-	i^+z^+	+	−	0.76	0.29	2.6
Sh I-4	i^+z^-	i^+z^+	+	−	1.1	0.98	1.1

The UV dose was 40 sec; P1 helper phage, when present, was added to give a m.o.i. = 2 to 3. E (= enzyme) and O.D. were measured at 30-min intervals in treated and control cultures.

TABLE 2

EFFECT OF P1 SUPERINFECTION ON PRODUCTION OF GALACTOSIDASE AND OF TRANSDUCING ACTIVITY

Bacterial strain	Treatment UV	Treatment P1	Maximum enzyme, units/ml	Transducing activity/ml
3.0S0-13-4-1	+	−	11.2	5×10^3
	+	+	18.5	1×10^6
3.0S0-13-4-2	+	−	18	<100
	+	+	26.9	1×10^5
W4032-W-1	+	−	1.1	4×10^4
	+	+	1.2	8×10^5

TGA medium, and tested for constitutive galactosidase levels, comparing them with i^+z^+ and with i^-z^+ cells. Out of 407 cultures tested, 401 showed low enzyme levels like those of the parent culture; 5 had no enzyme and were probably *lac*$^-$, presumably having lost the P1*dl* prophage; only one out of 407 (or 0.25 per cent) was like i^-z^+ and was probably a constitutive recombinant homogenote.[7] We conclude that the constitutive enzyme present in unirradiated cultures of i^+ heterogenotes is not produced by a minority of i^- cells, but is due to a certain degree of escape of the phage-associated z^+ gene from repression by a chromosomal i^+ gene.

The effect of λ prophage on UV-provoked derepression: When an i^+ heterogenote strain carrying P1*dl* z^+ also carries a prophage λ, which is readily induced to vegetative replication and maturation by small doses of UV light, the UV-induced synthesis of galactosidase is almost fully suppressed. Data for strains of the W-5 type are given in Table 3; other strains behave similarly. Note that the production of *lac*$^+$ transducing particles is also reduced. Development of phage λ after irradiation is fairly rapid, lysis beginning at 60 min. and being complete by 100 min.[8] The supression of both galactosidase synthesis and production of transducing

TABLE 3

EFFECT OF λ PROPHAGE ON UV DEREPRESSION OF GALACTOSIDASE IN HETEROGENOTES

Bacterial strain	Treatment UV	Treatment P1	Enzyme units/ml	Transducing activity/ml
W4032-W-5(λ)	+	−	2.9	4.4×10^3
	+	+	2.6	9.6×10^3
	−	+	1.0	9.8×10^4
	−	−	1.3	1.4×10^3
W4032-W-5λ^s	+	−	38.8	2.1×10^5
(λ-cured)	+	+	29.7	1.6×10^6
	−	+	0.77	3.6×10^3
	−	−	0.85	1.8×10^3
W4032-W-5(λ)	+	−	0.83	6.5×10^3
(λ-reinfected)	+	+	0.58	2.2×10^4
	−	+	0.87	4.0×10^3
	−	−	0.75	1.2×10^3

Before and after treatment the bacterial cells were grown in tryptone-yeast extract broth. Enzyme and transducing activity (on strain 2.0S0 as recipient) were measured 3 hr after treatment.

P1*dl* phage may be due to a general interference with a variety of biosynthetic processes by the developing phage λ.

Derepression following infection with a virulent mutant of phage P1: Confirmatory evidence that the partial derepression of the z^+ gene following UV treatment of heterogenotes reflects the removal of immunity, rather than some unknown action of UV light, was provided by the observation that derepression also follows another kind of immunity release. A virulent mutant for phage P1, called P1*kc vir*, was available, which upon infecting P1-lysogenic bacteria causes a large proportion of them to lyse and to release phage. An experiment in which cells of a heterogenote strain were superinfected with phage P1*kc vir* is presented to Figure 3. Production of galactosidase, in amounts corresponding to the complement for 2×10^6 fully-induced cells, was produced by infection of about 5×10^7 cells. Similar results were obtained with all heterogenote strains. With *E. coli* heterogenotes, most of the enzyme was released from the cells by 90 min. after superinfection, in parallel with release of phage; with *Shigella* heterogenotes, the release was slower. Note in Figure 3 that the lysate also contained transducing activity, the amount being about 1 transduction per 1,000 active phages. This indicates that the P1*dl* phage, whose z^+ gene controls the derepressed enzyme synthesis, participated in the phage yield. Control experiments showed no derepression of a chromosomally located z^+ gene in P1-sensitive or P1-lysogenic bacteria superinfected by P1*kc vir*. The presence of a λ prophage did not alter the results of superinfection with P1*kc vir*.

Discussion.—Phage P1 in the prophage state is partially inducible to vegetative replication, maturation and lysis by UV irradiation; 5 to 20 per cent of P1-lysogenic cells release phage after suitable UV-treatment. The UV provoked derepression of galactosidase synthesis under the control of a P1*dl* z^+ prophage is probably analogous to the derepression of phage genes that would lead to phage production from a normal P1 prophage. Likewise, superinfection with the virulent mutant P1*kc vir* overcomes prophage immunity and derepresses the function of genes in the prophage.

Most or all of the enzyme made constitutively after derepression by UV light is released as free enzyme into the medium. This indicates that most and possibly all the cells that produce enzyme also undergo lysis. The derepression of the z^+ gene is accompanied by derepression of the phage genes of P1*dl* that control the lytic process. There is apparently no "abortive" derepression of the *lac* genes

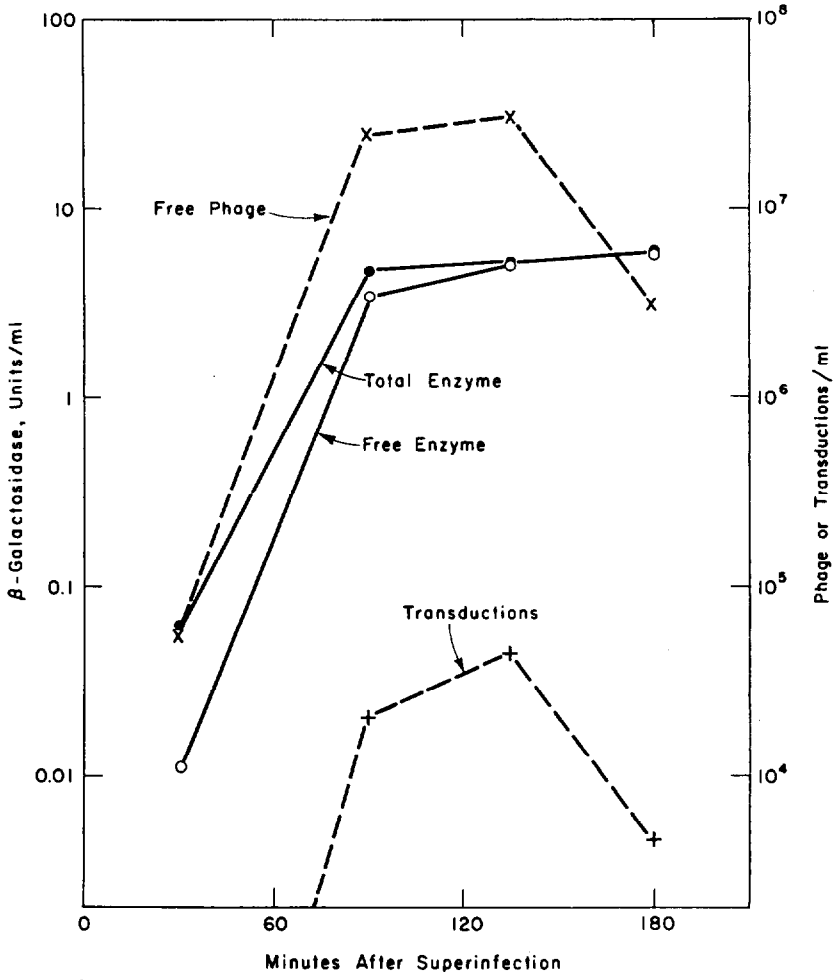

FIG. 3.—Production of galactosidase by *E. coli* strain 3.0S0-13-4-1 after multiple infection with phage P1*kc vir*.

analogous to the abortive transduction, in which the z^+ genes in P1*dl* phage function but lysis and enzyme release are not observed.[5]

The remarkable observation is that the effectiveness of repression mechanisms on the z^+ gene in phage depends on the state of the phage element. (*a*) In a newly-entered P1*dl* phage, the z^+ gene is subject to full repression by an i^+ gene in cis, to partial repression by i^+ in trans, and to variable degrees of repression by P1-immunity. (*b*) In an established P1*dl* prophage, the z^+ gene is subject to variable but almost complete repression by i^+, whether in cis or trans, and is insensitive to P1-immunity. (*c*) In a P1*dl* element that has been activated, by UV or by P1*kc vir*, the z^+ gene is again partially relieved from i^+ repression. A significant conclusion that seems justified by these findings is that the various repression mechanisms must act at the gene level, as has already been postulated on the basis of other evidence.[9] It is difficult to see how repression mechanism acting at the level of the protein-forming system—messenger-ribosome level—could discriminate

between messages coming from genes in the chromosome, or in a loose phage, or in a prophage.

The working of the repressive controls can hardly be speculated upon on the basis of present data. Since P1-immunity, which suppresses multiplication of a newly arrived phage and also depresses the function of the z^+ gene in such a phage, does not affect it in an established phage, it may be that P1-immunity *per se* is not the primary mechanism that supresses the function of most phage genes in the prophage state. A similar conclusion has recently been proposed on different grounds.[10]

We have no evidence as to whether the derepression of z^+ after UV-treatment on P1*dl* heterogenotes simply reflects a change in state of the prophage or is a result of multiplication of P1*dl*. In the case of λ*dg*-carrying heterogenotes, there is evidence that λ*dg* does not multiply unless a normal phage λ is also present;[11] yet, derepression of the *gal* genes occurs in the absence of normal λ. Hence, multiplication of the *gal*-containing element is apparently not needed.

Clearly, topographical considerations play a role in regulatory mechanisms, either because of variable local concentrations of repressors or because of neighborhood relations between genes. An illustration of the importance of such neighborhood relations is the derepression of chromsomal *gal*$^+$ genes upon activation of an adjacent λ prophage by UV treatment. In analogous experiments with P1-lysogenic bacteria there is no derepression of the chromsomal z^+ gene. All available evidence indicates that a P1 or P1*dl* prophage, if it is attached to any chromosomal site at all,[12] is not near the *lac* locus.[13] The chromosomal *gal* genes and the λ prophage may be considered as part of a superoperon, some of whose component genes become repressed or derepressed in a coordinate fashion; *lac* genes and phage genes in P1*dl* may constitute another such superoperon.

Summary.—UV-irradiation or infection by virulent phage mutant P1*kc vir* cause heterogenote strains carrying P1*dl* z^+ phage to produce some β-D-galactosidase in the absence of an external inducer. The enzyme produced in this derepressed synthesis is released free into the medium by lysis. The derepressed synthesis is attributed to the induction of the prophage that carries the z^+ gene. No derepression of chromosomal z^+ gene is observed when similar treatments are used on bacteria carrying a normal P1 prophage.

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